Phytochemistry, Vol. 26, No. 12, pp. 3233–3234, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
© 1987 Pergamon Journals Ltd.

BIOTRANSFORMATION OF ANHYDROVINBLASTINE TO VINBLASTINE BY A CELL-FREE EXTRACT OF *CATHARANTHUS ROSEUS* CELL SUSPENSION CULTURES

TSUYOSHI ENDO,* ANNE GOODBODY, JOHN VUKOVIC and MASANARU MISAWA

Allelix Inc. 6850 Goreway Drive, Mississauga, Ontario, Canada L4V 1P1

Key Word Index—*Catharanthus roseus*; Apocynaceae; tissue culture; enzymatic synthesis; anticancer agent; indole alkaloids; vinblastine; 3′,4′-anhydrovinblastine.

Abstract—A crude enzyme preparation obtained from cell suspension culture of *Catharanthus roseus* converted 3′,4′-anhydrovinblastine to vinblastine, an anticancer agent. NADH and $MnCl_2$ in the reaction mixture enhanced vinblastine yields.

INTRODUCTION

Vinblastine (VLB) and vincristine (VCR) are now well accepted in the treatment of various cancers in man. These medicinally important alkaloids are obtained only from leaves of *Catharanthus roseus*, and the yields are extremely low. Consequently, these drugs are among the most expensive compounds on the pharmaceutical market.

We have been investigating the efficient production of VLB type alkaloids with enzymes from cell suspension cultures of *C. roseus*. It has been demonstrated that 3′,4′-anhydrovinblastine (AVLB) can be obtained by coupling of vindoline and catharanthine using enzymes from cell suspension cultures with yields of 50% [1,2]. In order to synthesize VLB from AVLB, we have undertaken an investigation on the enzymatic transformation. McLauchlan *et al.* [3] reported that cell free extracts of *C. roseus* cell suspension culture could convert AVLB to VLB, but the rate of the conversion was low (0.57%). Here we present remarkably improved yields through the use of NADH.

RESULTS AND DISCUSSION

Preliminary experiments showed that the enzyme activity which converted AVLB to VLB is present in both leaves and cell suspensions of *C. roseus*. Identity of VLB formed was confirmed by UV spectrum, R_t in HPLC (using the conditions described in our previous paper [1]), and low and high resolution mass spectra.

The effects of various cofactors on the AVLB transformation catalysed by the enzyme from cell suspension cultures were examined. Compounds tested were vitamin B12, NAD(P) (H), FMN, and pyridoxal 5-phosphate. Of these compound NADH and NADPH gave detectable amounts of VLB. Further studies showed that multiple additions of NADH gave the best yields (Table 1). The effects of metal ions were also tested, and Mn^{2+} was found to be most effective for VLB formation (Table 2). The Optimum pH was pH 7.5 (data not shown). The highest rate of conversion so far obtained was 13% (using 200μg AVLB.H_2SO_4, 25.4μg VLB calculated as VLB.H_2SO_4 was formed in 5 hr by 2.7 mg protein under the conditions described in the Experimental sections.). Higher concen-

Table 1. Effect of NADH on VLB formation

Reaction mixture*	Conversion yield (%)
Complete	12.3
−NADH	N.D.
−Enzyme	1.8

*Composition of the reaction mixture and the method of addition of NADH were described in the text. Incubation; 5 h.
N.D. = not detected.

Table 2. Effects of the metal ions on VLB formation

Ion	Concentration (mM)	Activity (% of control)
Control*	—	100
$CaCl_2$	10	91
$MgSO_4$	10	108
$FeSO_4$	1	N.D.
$FeSO_3$	1	N.D.
$CoCl_2$	1	102
$ZnSO_4$	1	45
$CuSO_4$	1	98
$NiCl_2$	1	75
$MnCl_2$	1	152

*Composition of the reaction mixture and the method of addition of NADH were described in the text; in the control, 6.4% of AVLB incubated was converted to VLB.
Incubation; 3 hr.
N.D. = not detected.

*Present address: Research Center for Cell and Tissue Culture, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan.

trations of enzyme and longer incubations did not enhance the yield of VLB. Significant amounts of leurosine and an unknown alkaloid of molecular weight 824 (not catharine) were also formed as byproducts. The requirement for NADH suggests that the biotransformation of AVLB to VLB is not a single-step hydration. As Mangeney *et al.* [4] proposed, 4'-deoxyleurosidine or an iminium type intermediate may be involved in this reaction.

EXPERIMENTAL

Chemicals. AVLB·H_2SO_4 and leurosine were obtained from Dr J.P. Kutney, Department of Chemistry, University of British Columbia, B.C., Canada.

Extraction of enzyme. The cell line used was described in our previous report [1]. Two-week-old cells were frozen in liquid N_2 and stored at −20°C untill used. The enzyme was extracted from the cells with two vol. of Tris–HCl (pH 7.5, 100 mM) buffer, an equivalent wt of polyvinylpolypyrollidine, and 10 mM mercaptoethanol. The protein was pptd from 70% satd $(NH_4)_2SO_4$ soln. The pellet obtained was dissolved in the Tris–HCl buffer and desalted. All manipulations were performed at 0–4°C.

Reaction conditions. The reaction mixture consisted of 1.5 ml Tris–HCl buffer (pH 7.5, 100 mM), 1 mM $MnCl_2$, 0.2 mg AVLB.H_2SO_4, and *ca* 2 mg protein. The incubation was carried out at 30° for 3–5 h. During the incubation 0.5 mg NADH was added to the mixture every 30 min.

Alkaloid extraction and assay. The reaction was stopped by adding 0.1 ml 28% $(NH_4)OH$, and the alkaloid was extracted with EtOAc. VLB formed was quantified with TLC (solvent system, toluene–Me_2CO–MeOH–28% $(NH_4)OH$, 56:20:4:1) scanned by a TLC scanner (Schimadzu) at 280 nm.

Acknowledgement—We thank Dr J.P. Kutney, Department of Chemistry, University of British Columbia for MS measurements.

REFERENCES

1. Misawa, M., Endo, T., Goodbody, A., Vukovic, J., Chapple, C., Choi, L. and Kutney, J.P. (1987) *Phytochemistry* (in press).
2. Endo, T., Goodbody, A., Vukovic, J. and Misawa, M. (1987) *Phytochemistry* (in press).
3. McLauchlan, W.R., Hasan, M., Baxter, R.L. and Scott, A.I. (1983) *Tetrahedron* **39**, 3777.
4. Mangeney, P., Andriamialisoa, R.Z., Langlois, N., Langloiois, Y. and Potier, P. (1979) *J. Am. Chem. Soc.* **101**, 2243.